In re Application of: Ehud GAZIT
Serial No.: 10/562,852
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Examiner: DUTT, Aditi
Group Art Unit: 1649
Attorney Docket: 31230

In the Specification:

Please amend the title on **Page 1, line 1** of the specification as follows:

~~PEPTIDES ANTIBODIES DIRECTED THEREAGAINST AND METHODS USING SAME FOR DIAGNOSING AND TREATING AMYLOID ASSOCIATED DISEASES~~

PEPTIDES AND METHODS USING SAME FOR DIAGNOSIS AND TREATMENT OF AMYLOID-ASSOCIATED DISEASE

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Please amend the Paragraph beginning at **Page 44, line 10**, as follows:

To facilitate complex detection, the peptides of the present invention are highlighted preferably by a tag or an antibody. It will be appreciated that highlighting can be effected prior to, concomitant with or following aggregate formation, depending on the highlighting method. As used herein the term "tag" refers to a molecule, which exhibits a quantifiable activity or characteristic. A tag can be a fluorescent molecule including chemical fluorescers such as fluorescein or polypeptide fluorescers such as the green fluorescent protein (GFP) or related proteins (www.dotclontech.dotcom). In such case, the tag can be quantified via its fluorescence, which is generated upon the application of a suitable excitatory light. Alternatively, a tag can be an epitope tag, a fairly unique polypeptide sequence to which a specific antibody can bind without substantially cross reacting with other cellular epitopes. Such epitope tags include a Myc tag, a Flag tag, a His tag, a leucine tag, an IgG tag, a streptavidin tag and the like.

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Please amend the paragraphs beginning on **Page 10, line 29 – Page 17, line 10** of the specification as follows:

FIGs. 3a-c are schematic illustrations of a primary sequence comparison between human and rodent IAPP and the synthetic peptides of the present invention. Figure 3a is a sequence alignment of human and rodent IAPP (SEQ ID NOs: 152-153). A block indicates a seven amino acid sub-sequence illustrating the major inconsistencies between the sequences. The "basic amyloidogenic unit" is presented by bold letters and underlined. Figure 3b illustrates the chemical structure of the wild type IAPP peptide (SEQ ID NO: 1). Figure 3c illustrates the primary sequences and SEQ ID NOs.: 1-6 of the peptides derived from the basic amyloidogenic unit.

FIGs. 4a-b are graphs illustrating light absorbance at 405 nm as a function of time during fibril formation thus reflecting the aggregation kinetics of IAPP-derived peptides. The following symbols are used: closed squares – N1A, opened circles - G3A, closed circles – wild type, opened triangles - L6A, opened squares – I5A and closed triangles – F2A.

FIG. 5 is a histogram depicting mean particle size of assembled IAPP peptide and derivatives as measured by light scattering. Each column represents the results of 3-5 independent measurements.

FIGs. 6a-n are photomicrographs illustrating Congo Red binding to pre-assembled IAPP peptides. Normal field and polarized field micrographs are shown respectively for each of the following aged peptide suspensions: N1A peptide (SEQ ID NO: 2, Figures 6a-b), F2A peptide (SEQ ID NO: 3, Figures 6c-d), G3A peptide (SEQ ID NO: 4, Figures 6e-f), wild type peptide (SEQ ID NO: 1, Figures 6g-h), I5A peptide (SEQ ID NO: 5, Figures 6i-j) and L6A (SEQ ID NO: 6, Figures 6k-l). Buffer with Congo red reagent was used as a negative control visualized with and without polarized light as shown in Figures 6m and 6n, respectively.

FIGs. 7a-f are electron micrographs of "aged" IAPP peptide and derivatives. N1A peptide (SEQ ID NO: 2, Figure 7a), F2A peptide (SEQ ID NO: 3, Figure 7b), G3A peptide (SEQ ID NO: 4, Figure 7c), wild type peptide (SEQ ID NO: 1, Figure

7d), I5A peptide (SEQ ID NO: 5, Figure 7e) and L6A (SEQ ID NO: 6, Figure 7f). The indicated scale bar represents 100 nm.

FIG. 8a is a nucleic acid sequence alignment of wild type hIAPP (SEQ ID NO: 1) and a corresponding sequence modified according to a bacterial codon usage. Modified bases are underlined.

FIG. 8b is a schematic illustration of the pMALc2x-NN vector which is used for cytoplasmic expression of the 48 kDa MBP-IAPP protein. The V8 Ek cleavage site and the $(His)_6$ tag are fused C-terminally to the *malE* tag vector sequence. A factor *Xa* cleavage site for removal of the MBP tag is indicated.

FIG. 9 is a protein gel GelCode Blue staining depicting bacterial expression and purification of MBP and MBP-IAPP fusion protein. Bacterial cell extracts were generated and proteins were purified on an amylose resin column. Samples including 25 μg protein were loaded in each of Lanes 1-3 whereas 5 μg protein were loaded on each of lanes 4-5. Proteins were resolved on a 12 % SDS-PAGE and visualized with GelCode Blue staining. A molecular weight marker is indicated on the left. Lane 1 – 0.5 mM IPTG-induced soluble extract of MBP. Lane 2 – 0.1 mM IPTG-induced soluble extract of MBP-IAPP. Lane 3 – 0.5 mM IPTG-induced soluble extract of MBP-IAPP. Lane 4 – purified MBP. Lane 5 – purified MBP-IAPP. An arrow marks the MBP-IAPP.

FIGs. 10a-b are a dot-blot image (Figure 10a) and densitometric quantitation thereof (Figure 10b) depicting putative amyloidogenic sequences in hIAPP (SEQ ID NO: 61-88).

FIG. 11 is a graphic illustration depicting light absorbance at 405 nm as a function of time during fibril formation thus reflecting the aggregation kinetics of IAPP-derived peptides (SEQ ID NOs. 14-19).

FIGs. 12a-f are photomicrographs illustrating Congo Red binding to pre-assembled IAPP peptides. Polarized field micrographs are shown for each of the following one day aged peptide suspensions: NFLVHSSNN peptide (SEQ ID NO: 14, Figure 12a), NFLVHSS (SEQ ID NO: 15, Figure 12b), FLVHSS (SEQ ID NO:

16, Figure 12c), NFLVH (SEQ ID NO: 17, Figure 12d), FLVHS (SEQ ID NO: 18, Figure 12e) and FLVH (SEQ ID NO: 19, Figure 12f).

FIGs. 13a-f are electron micrographs of "aged" IAPP peptides. NFLVHSSNN peptide (SEQ ID NO: 14, Figure 13a), NFLVHSS (SEQ ID NO: 15, Figure 13b), FLVHSS (SEQ ID NO: 16, Figure 13c), NFLVH (SEQ ID NO: 17, Figure 13d), FLVHS (SEQ ID NO: 18, Figure 13e) and FLVH (SEQ ID NO: 19, Figure 13f). The indicated scale bar represents 100 nm.

FIGs. 14a-f are graphs showing secondary structures in the insoluble IAPP aggregates as determined by Fourier transformed infrared spectroscopy. NFLVHSSNN peptide (SEQ ID NO: 14, Figure 14a), NFLVHSS (SEQ ID NO: 15, Figure 14b), FLVHSS (SEQ ID NO: 16, Figure 14c), NFLVH (SEQ ID NO: 17, Figure 14d), FLVHS (SEQ ID NO: 18, Figure 14e) and FLVH (SEQ ID NO: 19, Figure 14f).

FIG. 15 is a chemical structure of a previously reported amyloidogenic peptide fragment of Medin [Haggqvist (1999) Proc. Natl. Acad. Sci. U S A 96:8669-8674].

FIGs. 16a-b are graphs illustrating light absorbance at 405 nm as a function of time during fibril formation thus reflecting the aggregation kinetics of Medin-derived peptides (SEQ ID NOs: 21-25, 154-155). Figure 16a illustrates a short-term kinetic assay. Figure 16b illustrates a long-term kinetic assay .

FIGs. 17a-f are electron micrographs of "aged" Medin-derived peptides. NFGSVQFA (SEQ ID NO: 156)- Figure 17a, NFGSVQ (SEQ ID NO: 21) - Figure 17b, NFGSV (SEQ ID NO: 22) - Figure 17c, FGSVQ (SEQ ID NO: 23) - Figure 17d, GSVQ (SEQ ID NO: 24) - Figure 17e and FGSV (SEQ ID NO: 25) - Figure 17f. The indicated scale bar represents 100 nm.

FIGs. 18a-f are photomicrographs illustrating Congo Red binding to pre-assembled Medin-derived peptides. Polarized field micrographs are shown for each of the following aged peptide suspensions: NFGSVQFA (SEQ ID NO: 156) - Figure 18a, NFGSVQ (SEQ ID NO: 21) - Figure 18b, NFGSV (SEQ ID NO: 22) - Figure

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18c, FGSVQ (SEQ ID NO: 23) - Figure 18d, GSVQ (SEQ ID NO: 24) - Figure 18e and FGSV (SEQ ID NO: 25) - Figure 18f.

FIGs. 19a-c depict the effect of an alanine mutation on the amyloidogenic features of the hexapeptide amyloidogenic fragment of Medin. Figure 19a – is a graph illustrating light absorbance at 405 nm as a function of time during fibril formation thus reflecting the aggregation kinetics of Medin-derived alanine mutant (SEQ ID NOs: 21, 26); Figure 19b is an electron micrograph of "aged" Medin-derived alanine mutant, The scale bar represents 100 nm; Figure 19c – is a photomicrograph illustrating Congo Red binding to pre-assembled Medin-derived peptide mutant.

FIGs. 20a-b are the amino acid sequence of human Calcitonin (Figure 20a, SEQ ID NOs: 158 and 179) and chemical structure of an amyloidogenic peptide fragment of human Calcitonin (Figure 20b). Underlined are residues 17 and 18 which are important to the oligomerization state and hormonal activity of Calcitonin [Kazantzis (2001) Eur. J. Biochem. 269:780-791].

FIGs. 21a-d are electron micrographs of "aged" Calcitonin-derived peptides. DFNKF (SEQ ID NO: 27) - Figure 21a, DFNK (SEQ ID NO: 29) - Figure 21b, FNKF (SEQ ID NO: 28) - Figure 21c and DFN (SEQ ID NO: 30) - Figure 21d. The indicated scale bar represents 100 nm.

FIGs. 22a-d are photomicrographs illustrating Congo Red binding to pre-assembled Calcitonin-derived peptides. Polarized field micrographs are shown for each of the following aged peptide suspensions: DFNKF (SEQ ID NO: 27) - Figure 22a, DFNK (SEQ ID NO: 29) - Figure 22b, FNKF (SEQ ID NO: 28) - Figure 22c and DFN (SEQ ID NO: 30) - Figure 22d.

FIG. 23 is a graphic illustration showing secondary structures in the insoluble Calcitonin aggregates as determined by Fourier transformed infrared spectroscopy (SEQ ID NOs: 27-30).

FIGs. 24a-c depict the effect an alanine mutation on the amyloidogenic features of the pentapeptide amyloidogenic fragment of Calcitonin (SEQ ID NO: 31).

Figure 24a is an electron micrograph of "aged" Calcitonin-derived alanine mutant. The scale bar represents 100 nm; Figure 24b – is a photomicrograph illustrating Congo Red binding to pre-assembled Calcitonin-derived peptide mutant; Figure 24c is a graph showing secondary structures in the mutant peptide as determined by Fourier transformed infrared spectroscopy.

FIG. 25 is an electron micrograph depicting self-assembly of "aged" Lactotransferrin-derived peptide (SEQ ID NO: 32). The scale bar represents 100 nm.

FIG. 26 is an electron micrograph depicting self-assembly of "aged" Serum amyloid A protein-derived peptide (SEQ ID NO: 33). The scale bar represents 100 nm.

FIG. 27 is an electron micrograph depicting self-assembly of "aged" BriL-derived peptide (SEQ ID NO: 34). The scale bar represents 100 nm.

FIG. 28 is an electron micrograph depicting self-assembly of "aged" Gelsolin-derived peptide (SEQ ID NO: 35). The scale bar represents 100 nm.

FIG. 29 is an electron micrograph depicting self-assembly of "aged" Serum amyloid P-derived peptide (SEQ ID NO: 36). The scale bar represents 100 nm.

FIG. 30 is an electron micrograph depicting self-assembly of "aged" Immunoglobulin light chain-derived peptide (SEQ ID NO: 37). The scale bar represents 100 nm.

FIG. 31 is an electron micrograph depicting self-assembly of "aged" Cystatin C-derived peptide (SEQ ID NO: 38). The scale bar represents 100 nm.

FIG. 32 is an electron micrograph depicting self-assembly of "aged" Transthyretin-derived peptide (SEQ ID NO: 39). The scale bar represents 100 nm.

FIG. 33 is an electron micrograph depicting self-assembly of "aged" Lysozyme-derived peptide (SEQ ID NO: 40). The scale bar represents 100 nm.

FIG. 34 is an electron micrograph depicting self-assembly of "aged" Fibrinogen-derived peptide (SEQ ID NO: 41). The scale bar represents 100 nm.

FIG. 35 is an electron micrograph depicting self-assembly of "aged" Insulin-derived peptide (SEQ ID NO: 42). The scale bar represents 100 nm.

FIG. 36 is an electron micrograph depicting self-assembly of "aged" Prolactin-derived peptide (SEQ ID NO: 43). The scale bar represents 100 nm.

FIG. 37 is an electron micrograph depicting self-assembly of "aged" Beta 2 microglobulin-derived peptide (SEQ ID NO: 44). The scale bar represents 100 nm.

FIG. 38 is a graphic representation of the effect of an inhibitory peptide on IAPP self-assembly (SEQ ID NO: 45). Squares – wild type (wt) IAPP peptide; triangles – wt-IAPP + inhibitory peptide; circles – no peptides.

FIG. 39 is a graphic illustration depicting light absorbance at 405 nm as a function of time during fibril formation thus reflecting the aggregation kinetics of IAPP-derived peptides (SEQ ID NOs. 46-49 and 89).

FIG. 40 is a histogram representation illustrating turbidity of IAPP analogues following seven day aging (SEQ ID NOs. 46-49).

FIG. 41a-f are electron micrographs of "aged" IAPP analogues. NFGAILSS (SEQ ID NO: 46)- Figure 41a; NFGAILSS (SEQ ID NO: 46) - Figure 41b; NIGAILSS (SEQ ID NO: 47) - Figure 41c; NLGAILSS (SEQ ID NO: 48) - Figure 41d; NVGAILSS (SEQ ID NO: 49) - Figure 41e and NAGAILSS (SEQ ID NO: 89, 91, 92) - Figure 41f. The indicated scale bar represents 100 nm.

FIGs. 42a-c illustrate the binding of IAPP- NFGAILSS to analogues of the minimal amyloidogenic sequence SNNXGAILSS (SEQ ID NO: 90, X = any natural amino acid but cysteine). Figure 42a shows short exposure of the bound peptide-array. Figure 42b shows long exposure of the bound peptide-array. Figure 42c shows quantitation of the short exposure (Figure 42a) using densitometry and arbitrary units (SEQ ID NOs: 91-110).

FIG. 43a is a Ramachandran plot showing the sterically allowed regions for all residues (yellow for fully allowed, orange for partially allowed), for L-Proline (blue) and for the achiral Aib residue (magenta).

FIGs. 43b-c are schematic illustrations showing the chemical structure of the longer wild-type IAPP peptide (ANFLVH, SEQ ID NO: 124, Figure 43b) and the Aib modified structure thereof peptide (Aib-NF-Aib-VH, SEQ ID NO: 125, Figure 43c).

Functional groups suitable for modicifcation are marked in blue (Figure 43b) while modified groups are marked in red (Figure 43c).

FIGs. 44a-d are electron micrographs of "aged" IAPP analogues. Figure 44a – ANFLVH (SEQ ID NO: 124); Figure 44b – ANFLV (SEQ ID NO: 126); Figure 44c - Aib-NF-Aib-VH (SEQ ID NO: 125); and Figure 44d - Aib-NF-Aib-V (SEQ ID NO: 127). The indicated scale bar represents 100 nm.

FIGs. 45a-d are photomicrographs illustrating Congo Red binding to pre-assembled wild type and Aib modified IAPP peptides. Polarized field micrographs are shown for each of the following aged (i.e., 11 days) peptide suspensions. Figure 45a – ANFLVH (SEQ ID NO: 124); Figure 45b – ANFLV (SEQ ID NO: 126); Figure 45c - Aib-NF-Aib-VH (SEQ ID NO: 125); Figure 45d - Aib-NF-Aib-V (SEQ ID NO: 127).

FIGs. 46a-b are graphs showing secondary structures in the insoluble wild type and Aib modified hIAPP aggregates as determined by Fourier transformed infrared spectroscopy (FT-IR). Figure 46a – wild-type peptide ANFLVH (SEQ ID NO: 124) and the corresponding Aib modified peptide (SEQ ID NO: 125) as designated by arrows. Figure 46b - wild-type ANFLVH (SEQ ID NO: 126) and the corresponding Aib modified peptide (SEQ ID NO: 127) as designated by ~~errows~~arrows.

FIG. 47 is a graph showing the inhibitory effect of Aib modified peptides on amyloid fibril formation. Wild type IAPP (SEQ ID NO: 1) was incubated alone or with the various peptides of the present invention (SEQ ID NO: 125, 127 and 157). Fibril formation as a function of time was determined using ThT fluorescence.

FIG. 48 is a histogram showing the inhibitory effect of short aromatic sequences (SEQ ID NOs. 112-123) on IAPP self-assembly.

FIGs. 49a-d are graphs depicting iterative cycles of selection of IAPP fibrilization inhibitors. Fibrilization was monitored by ThT fluorescence assay. Fluorescence values of IAPP alone (4 μM) or in the presence of assayed compounds (40 μM) were tested. Measurements were taken once IAPP fluorescence reached a plateau. IAPP fluorescence was arbitrary set as 100. Figure 49a shows the results of

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the first round of selection of IAPP fibrilization inhibitors. EG1 =D-Phe-D-Phe-D-Pro (SEQ ID NO: 128); EG2 = Aib-D-Phe-D-Asn-Aib (SEQ ID NO: 129); EG3 =D-Phe-D-Asn-D-Pro (SEQ ID NO: 130); EG4 =Aib-Asn-Phe-Aib (SEQ ID NO: 131); EG5 = Gln-Lys-Leu-Val-Phe-Phe (SEQ ID NO: 132); EG6 =Tyr-Tyr (SEQ ID NO: 133); EG7 = Tyr-Tyr-NH2 (SEQ ID NO: 112); EG8 =Aib-Phe-Phe (SEQ ID NO: 113). Figure 49b shows the results of the second round of selection of IAPP fibrilization inhibitors. EG13=Asn-Tyr-Aib (SEQ ID NO: 118); EG14=Asn-Tyr-Pro (SEQ ID NO: 119); EG15=D-Pro-D-Tyr-D-Asn (SEQ ID NO: 120); EG16=D-Tyr-Aib SEQ ID NO: 121); EG17=D-Pro-D-Tyr (SEQ ID NO: 122); EG18=D-Tyr-D-Pro (SEQ ID NO: 123). Figure 49c shows the results of the third round of selection of IAPP fibrilization inhibitors. d-F-P=D-Phe-Pro (SEQ ID NO: 147); P-d-F=Pro-D-Phe (SEQ ID NO: 148); EG19=Asn-Tyr-Tyr-Pro (SEQ ID NO: 134); EG20=Tyr-Tyr-Aib (SEQ ID NO: 135); EG21=Aib-Tyr-Tyr (SEQ ID NO: 136); EG22=Aib-Tyr-Tyr-Aib (SEQ ID NO: 137); EG23=D-Asn-Tyr-Tyr-D-Pro (SEQ ID NO: 138). Figure 49d shows the results of the forth round of selection of IAPP fibrilization inhibitors. EG24=Pro-Tyr-Tyr (SEQ ID NO: 139); EG25=Tyr-Tyr-Pro (SEQ ID NO: 140); EG26= Pro-Tyr-Tyr-Pro (SEQ ID NO: 141); EG27= D-Tyr-D-Tyr (SEQ ID NO: 142); EG28= D-Pro-Aib (SEQ ID NO: 143); EG29= D-Phe-D-Pro (SEQ ID NO: 144); EG30=D-Trp-Aib (SEQ ID NO: 145); EG31= D-Trp-D-Pro (SEQ ID NO: 146).

FIG. 50 is a graph depicting Inhibition of Aβ (1-40) fibril formation by D-Trp-Aib (SEQ ID NO: 145). Aβ 1-40 stock solution was diluted to a final concentration of 5 μM in 100 mM NaCl, 10 mM sodium phosphate buffer (pH 7.4) with 10 μM D-Trp-Aib (triangles) or without any addition (squares). Fluorescence values were measured after addition of 0.3 μM Tht to each sample. The results represent the mean of two independent measurements.

FIGs. 51a-c are photomicrographs depicting the inhibitory effect of D-Trp-Aib (SEQ ID NO: 145)on the fibrilization of Aβ as visualized by TEM. Figure 51a shows Aβ alone. Figures 51b-c shows two different field of Aβ incubated in the presence of the inhibitor.